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Near-infrared Spectroscopy in the Routine Diagnostic Work-up of Patients With Leg Ischaemia

D.T. Ubbink[1,2]* and B. Koopman[1]

Departments of [1]Surgery, and [2]Clinical Epidemiology and Biostatistics, Academic Medical Center, Amsterdam, The Netherlands

Objective. *To examine the reproducibility and clinical applicability of near-infrared spectroscopy (NIRS) in patients with leg ischaemia.*
Design. *Prospective comparative diagnostic study.*
Materials and methods. *Routinely measured peripheral blood pressure and microcirculatory parameters were compared with tissue oxygen saturation (TsO_2) measurements using a new NIRS device on the calf muscle and the foot. Healthy subjects (n=20) and patients in different stages of leg ischaemia (n=45) were investigated at rest and after provocation: a treadmill test, arterial occlusion and a change in posture. Healthy volunteers were used as an age-matched reference population.*
Results. *Reproducibility of the NIRS was excellent (intraclass correlation coefficient at rest was 0.91 (95% confidence interval: 80–99). Resting TsO_2 (65%) in healthy controls did not differ significantly from that in patients (Fontaine 2: 66%; F3/4: 68%). After exercise, a significant reduction in TsO_2 was observed only in patients: Fontaine 1: 60%; Fontaine 2: 21%, Fontaine 3/4: 29%. The ankle/brachial index after exercise showed a good correlation (R=0.73) with TsO_2 at the end of the treadmill test. No correlation between NIRS and other micro- or macrocirculatory parameters was found.*
Conclusion. *NIRS is a very reproducible tool to assess tissue oxygen saturation, but is not useful for the routine work-up of patients with leg ischaemia.*

Key words: Spectroscopy, near-infrared; Peripheral vascular diseases; Reproducibility of results; Microcirculation; Oximetry.

Introduction

Near-infrared spectroscopy (NIRS) was introduced as a technique to determine the degree of tissue oxygenation several decades ago.[1] NIRS enables direct and noninvasive measurements of the tissue oxygen saturation (TsO_2) by examining absorption differences in the spectra of oxygen carriers, such as haemoglobin, myoglobin and cytochrome a, a_3.[2] This technique has been applied to study the (patho) physiology of skeletal muscle metabolism, for example during exercise,[3,4] in patients with diabetes or compartmental syndrome,[5,6] or to monitor perfusion during carotid endarterectomy.[7–9]

In patients with intermittent claudication, local oxygen supply to the calf muscles is reduced, for which NIRS might be a useful detection device.[10–12] Doppler blood pressure measurements and arterial duplex scanning allow for a snapshot assessment of the functional state of the arteries. In contrast, NIRS measurements enable direct and continuous observation of the TsO_2, even during exercise.[13–15] Previous studies demonstrate a correlation between oxygen desaturation during exercise and the severity of the vascular insufficiency. Thus, NIRS could give complementary information about the amount of tissue perfusion and improve the diagnostic investigation of this disease.

In patients with critical leg ischaemia the microcirculatory skin perfusion is significantly reduced, which ultimately results in tissue loss. Methods to assess skin microcirculation are transcutaneous oxygen pressure ($TcpO_2$) measurements, demonstrating the local oxygen tension at the surface of the skin, and laser Doppler fluxmetry (LDF), as a measure of the total skin perfusion.[16,17] These techniques are of additional value in the assessment of the severity and the indication for treatment of leg ischaemia.[18–21]

*Corresponding author. Dr D.Th. Ubbink, Department of Clinical Epidemiology and Biostatistics, J1B-215, Academic Medical Centre, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands.
E-mail address: d.ubbink@amc.uva.nl

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NIRS, measuring a different entity of the local perfusion, might also add information about this local microcirculation.

In this study we compared a new NIRS device with other (routinely) applied diagnostic investigations for leg ischaemia regarding its reproducibility and value in the routine diagnostic work-up of patients with various degrees of leg ischaemia.

Materials and Methods

Subjects and patients

Healthy volunteers were randomly approached or responded to a poster invitation to participate in this study. Eligible patients were those referred to the vascular laboratory to undergo routine arterial investigation. The patients' symptoms were graded according to the Fontaine classification. The study was approved by the hospital's medical ethics committee and all patients and subjects were enrolled after informed consent.

NIRS

A new NIRS device (Inspectra 325; Hutchinson Technology Inc., Hutchinson, MN, USA) was used with a 25 mm probe. This instrument uses second differential processing of a measured tissue attenuation spectrum, in combination with the emission of four wavelengths (680, 720, 760 and 800 nm), which should reduce tissue scattering effects and make TsO_2 insensible to path length differences. The emitted near-infrared light passes through the skin and the subcutaneous tissue into the muscle and is reflected and absorbed by haemoglobin. The amount of light absorbed is dependent on the redox state of the iron molecule in haemoglobin.[1] Ischaemia increases oxygen extraction, decreasing the oxyhaemoglobin/deoxyhaemoglobin ratio. Therefore, NIRS measures quantitatively and continuously the muscle or tissue sO_2. The putative advantage of NIRS in diagnosing insufficient tissue perfusion is its penetration depth, enabling the muscular compartments of the lower leg to be examined. Furthermore, the measurement is not affected by motion artefacts. Thus, tissue oxygen supply can be recorded continuously during exercise at the time patients experience their ischaemic pain. The probe of the NIRS device was attached to the skin via an adhesive holder. The device was nulled by putting the probe in the zeroing box while switching on the instrument and was calibrated subsequently. A 10 min warming-up period for the device was applied before starting the experiment.

Microcirculatory techniques

Transcutaneous oxygen pressure ($TcpO_2$: TCM400; Radiometer, Copenhagen) was measured after calibration at ambient oxygen pressure. The $TcpO_2$ electrode was heated to 44 °C. Thus, local blood flow is maximised to promote gas diffusion from the superficial microvessels to the electrode, in which the $TcpO_2$ is measured through a reduction–oxidation reaction and is expressed in mmHg.[22] Laser Doppler fluxmetry (LDF: Periflux 4000; Perimed, Stockholm) measures the perfusion of the superficial skin (1.5 mm) by quantifying the microvascular perfusion on the basis of the Doppler shift the emitted laser light undergoes when it is reflected by moving particles, mainly erythrocytes. This flux is expressed in volts.[16]

Macrocirculatory techniques

Both the ankle-brachial blood pressure index (ABPI) and the toe systolic blood pressure (TSBP) were assessed. The ankle blood pressure was measured by means of an 8-MHZ Doppler scanning probe (PV lab, Stöpler, Electric Diagnostic Instruments, Burbank, Ca,. USA) and a 12 cm ankle cuff, at the dorsal pedal and posterior tibial arteries. The brachial blood pressure was determined using an automatic blood pressure monitor (Dinamap Plus; Criticon, Tampa, Fl, USA). TSBP was measured at the hallux (TP1) by means of photoplethysmography (PV lab, Stöpler, Electric Diagnostic Instruments, Burbank, Ca,. USA) and a digital cuff of 1.5 or 2.5 cm in width, depending on the diameter of the toe ($\pm$120% of the hallux diameter).

Measurement protocol

To assess the reproducibility of the various diagnostic tests, the perfusion and blood pressures were measured in one leg, at rest, during exercise, and after a change in posture. All measurements were performed twice, in healthy subjects, by the same observer (BK), with a 2-week interval. The subjects were investigated in the supine position. The probe of the NIRS device and the $TcpO_2$ electrode were attached to their holders on the lateral side of the calf muscle area. Then, ABPI and TSBP were determined at rest. Next, a 5-min. exercise test on a treadmill was performed with a speed of 3.1 km/h and an 8% slope. $TcpO_2$ and NIRS recordings were performed continuously during the test. A typical recording of NIRS is shown in Fig. 1. Within 1 min after the exercise, the ABPI measurement was repeated. Subsequently, NIRS, LDF and $TcpO_2$ measurements were performed during provocation tests. The holders of the NIRS and $TcpO_2$ probes were moved to the dorsum of the foot in the first intermetatarsal space, while LDF was attached on the plantar side of the big toe. The $TcpO_2$ electrode was located distal to the NIRS holder. By inflating a cuff above the ankle, arterial occlusion was induced for 3 min. During the post-occlusive reactive hyperaemic response the overshoot of the tissue perfusion was measured (peak minus resting values). Finally, the subject changed from the supine to the sitting position, provoking a vasoconstriction response. This response was expressed as supine/sitting ratio.

In the diagnostic work-up substudy, patients underwent the same procedures as healthy subjects. In all cases, the most severely affected leg was investigated.

Statistics

To evaluate reproducibility, the intraclass correlation coefficient (ICC) (two-way mixed, absolute agreement) of the various parameters between both measurement sessions was assessed. The ICC can be arbitrarily interpreted as poor (<0.20), fair (0.21–0.40), moderate (0.41–0.60), good (0.61–0.80) and excellent (0.81–1.00).[20,23]

The diagnostic value of NIRS in leg ischaemia was analysed by evaluating the correlation of NIRS with other micro- and macrocirculatory parameters of arterial insufficiency, using the Pearson correlation coefficient. Furthermore, the differences in mean responses upon provocation tests between the various Fontaine classifications were evaluated by means of an unpaired Student *t*-test. All statistical analyses were performed using SPSS for Windows, version 12.0.2 (SPSS Inc., Chicago, Ill., USA).

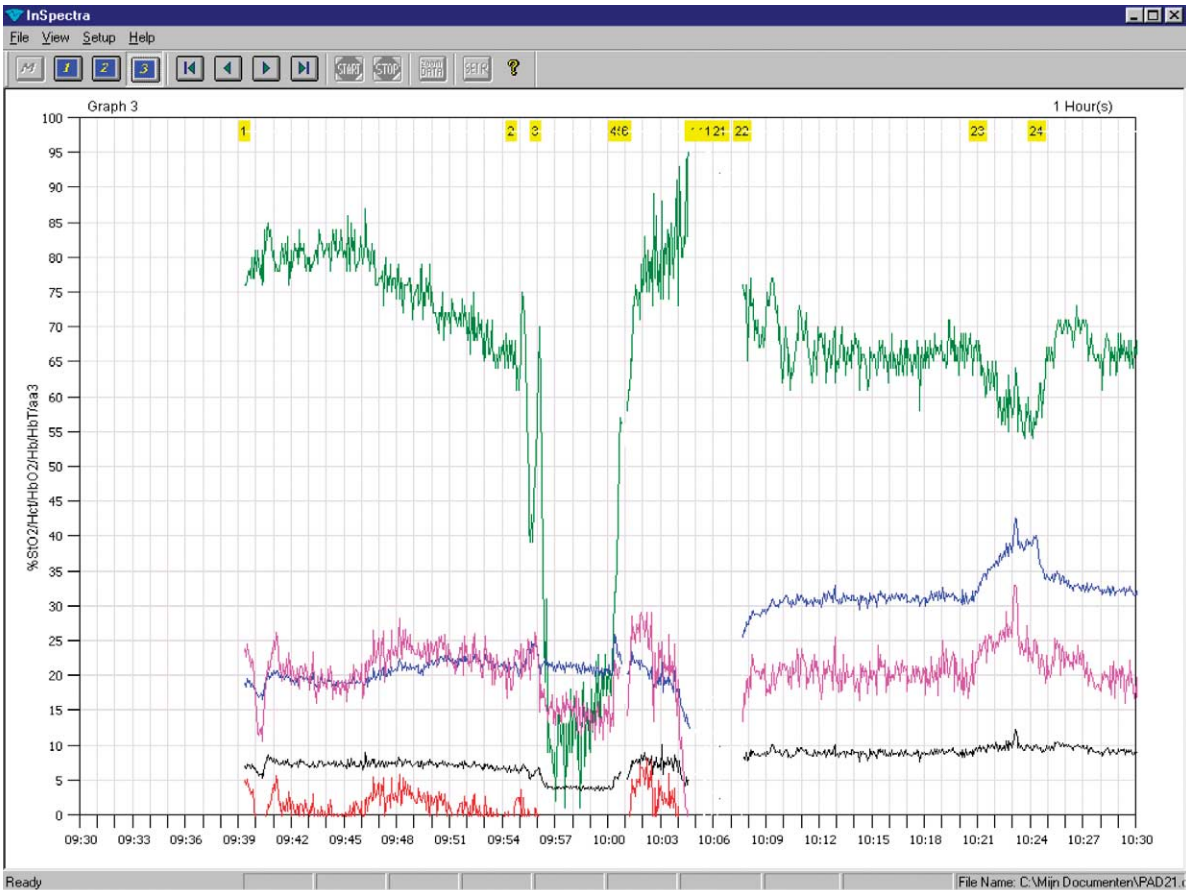

Fig. 1. Typical recording of NIRS in a patient with intermittent claudication. The green curve represents the TsO_2. A clear decrease can be seen in TsO_2 during the treadmill exercise.

Results

Reproducibility study

Twenty healthy volunteers, 11 males and 9 females, with a mean age of 30 years (range 21–55 years), participated. They had no walking difficulties or significant physical disorders. The mean interval between both investigation sessions was 15 days. All subjects had normal ABPIs above 100% (mean 115.6%). The reproducibility of NIRS on the calf, both at rest and after exercise, were excellent (ICCs of at least 0.90) and were higher than the other diagnostic tests (Table 1): $TcpO_2$ showed a lower ICC, which further decreased during exercise. The reproducibility of the systolic blood pressures of toe and ankle were 'good' and 'excellent', respectively, whereas the ICC of the ABPI was poor, both before and after exercise. This was due to the small range of ABPI values in these healthy subjects. Men had a strikingly higher TsO_2 than the women did (Table 1). This was found on the calf, but not on the dorsum of the foot.

Resting values of $TcpO_2$ and NIRS on the dorsum of the foot were moderately reproducible, while the reproducibility of the skin perfusion, as measured with LDF on the hallux, was good (Table 2). Furthermore, NIRS and LDF showed a clear postocclusive hyperaemic response and a vasoconstriction response, as opposed to $TcpO_2$. With NIRS, both provocation responses were moderately reproducible, whereas using LDF this was the case only with the vasoconstriction response. Both provocation tests could not be detected using $TcpO_2$, as the probe heats up the skin up to 44 °C causing active vasodilatation, which overrides any local microvascular response.

Diagnostic work-up study

Forty-four patients were investigated for the presence of arterial occlusive disease, comprising 19 males and 25 females. Their mean age was 60 years (range 34–78 years). In one of the female subjects both legs were measured, i.e. a total of 45 legs were investigated. The prevalence of common vascular risk factors was: smoking 38%, diabetes mellitus 38%, hypertension 42%, hyperlipidaemia 36% and a history of cerebro-cardiovascular disease 27%. Out of the 44 patients included, 16 presented with complaints of leg pain, not likely to be caused by leg ischaemia, and served as age-matched controls (Table 3). One subject in this group appeared to have asymptomatic peripheral vascular disease. Twenty patients had intermittent claudication and a decreased ABPI after the treadmill test (F2). Nine patients suffered from pain at rest (at night) with or without ulcerations (F3/4). The treadmill test was sufficient for all patients with vascular disease to provoke their usual complaints in the investigated leg. Four patients could not perform the treadmill test because of severe pain at rest.

The various tests showed significant differences between controls and patients with vascular disease (Table 3). ABPI and TSBP were useful indicators to determine the presence of leg ischaemia. $TcpO_2$, as measured on the calf, was able to discern critically ischaemic patients (F3/4). TsO_2 values from the NIRS measurements on the calf under resting conditions could not discriminate between the Fontaine stages. In response to treadmill exercise, NIRS measurements did show a significant difference in TsO_2 between controls and F2 and F3/4 patients, illustrating that the desaturation of haemoglobin in vascular patients was more pronounced during exercise. Under resting conditions, NIRS did not correlate with ABPI, but

Table 1. Mean values of the various parameters measured during the first (1) and second (2) measurement session in healthy controls and compared by means of an intraclass correlation coefficient (ICC, with 95% confidence intervals)

Measurements	Moment	Gender	1	2	ICC (95% CI)
ABPI (%)	Before treadmill		115.6	117.3	0.33*
	After treadmill		111.2	115.1	−0.44*
ASBP (mmHg)	Before treadmill		138.9	139.4	0.90 (0.75–0.96)
	After treadmill		142.6	142.5	0.89 (0.71–0.96)
TSBP (mmHg)			99.5	99.6	0.69 (0.21–0.88)
$TcpO_2$ (mmHg)	Before treadmill		64.9	64.6	0.49 (−0.35–0.80)
	End treadmill		72.2	70.1	0.25*
NIRS (TsO_2; %)	Before treadmill	Males	82.8	80.5	0.91 (0.77–0.96)
		Females	53.1	49.7	
	End treadmill	Males	77.3	74.8	0.90 (0.74–0.96)
		Females	47.6	49.8	

Measurements were recorded in the supine position with the probes of $TcpO_2$ and NIRS attached to the lateral side of the calf and were repeated after or at the end of the treadmill test. ABPI, ankle/brachial pressure index; ASBP, ankle systolic blood pressure; TSBP, toe systolic blood pressure; $TcpO_2$, transcutaneous oxygen pressure; NIRS, near-infrared spectroscopy; TsO_2, tissue oxygen saturation.

* Correlation not significantly different from zero.

Table 2. Mean values of the various parameters measured during the first (1) and second (2) measurement session in healthy controls

Measurements	Parameters	1	2	ICC (95% CI)
$TcpO_2$ (mmHg)	Rest	57.1	54.8	0.58 (−0.08–0.83)
	Peak-rest	−1.5	0.34	0.09*
	Supine/sitting ratio	1.08	1.11	0.53 (−0.25–0.81)
LDF (V)	Rest	0.103	0.110	0.70 (0.24–0.88)
	Peak-rest	0.726	0.463	−0.39*
	Supine/sitting ratio	0.17	0.21	0.46 (−0.29–0.78)
NIRS (TsO_2; %)	Rest	71	69	0.53 (−0.23–0.82)
	Peak-rest	10	10	0.52 (−0.24–0.81)
	Supine/sitting ratio	0.56	0.46	0.55 (−0.15–0.83)

Intraclass correlation coefficients (ICC) are given with 95% confidence intervals. Measurements were performed at rest in the supine position (except for the change in posture), with the probes of $TcpO_2$ and NIRS attached to the dorsum of the foot, and the LDF on the hallux. $TcpO_2$, transcutaneous oxygen pressure; LDF, laser Doppler fluxmetry; NIRS, near-infrared spectroscopy; TsO_2, tissue oxygen saturation.
* Correlation not significantly different from zero.

after exercise a lower ABPI was significantly correlated with a reduced TsO_2 ($R=0.73$; Fig. 2). The presence of diabetes mellitus appeared to be a confounding factor in this correlation: In non-diabetic patients the correlation was higher ($R=0.84$) than in diabetics ($R=0.60$). In addition, the decrease in TsO_2 during exercise (TsO_2 during exercise minus TsO_2 before exercise) showed a similar correlation with ABPI after exercise ($R=0.65$).

Resting values of NIRS and $TcpO_2$ on the dorsum of the foot and LDF on the hallux showed no correlation with the presence nor with the severity of leg ischaemia (Table 4). The postocclusive hyperaemia responses as measured by NIRS and LDF did not significantly differ between the various Fontaine stages. Only NIRS showed a significantly lower postural vasoconstriction response in patients than in controls. LDF after provocation tended to decrease with more severe leg ischaemia, but differences between the Fontaine stages were not significant. No correlation was apparent between resting NIRS and $TcpO_2$ or between resting NIRS and TSBP. Also, no correlation was found between LDF and NIRS regarding the hyperaemia or vasoconstriction responses.

Discussion

This study shows that NIRS appears to be a very reproducible tool to assess tissue oxygenation as compared with other clinical investigations used to diagnose leg ischaemia. However, we did not find NIRS useful to differentiate between the different stages of arterial insufficiency. We found an excellent reproducibility of NIRS when measuring TsO_2 in the calf region and a moderate reliability of TsO_2 on the dorsum of the foot, both at rest and during provocation tests. A small study of Kragelj *et al.*[24] also showed a moderate variability of TsO_2 measurements in postocclusive hyperaemic responses, but measurements at rest or during exercise were not performed. Most of the other macro-and microcirculatory parameters used in the present study were also reproducible and in agreement with an earlier study by De Graaff *et al.*[20] However, they found a much better reproducibility of the ABPI (ICC of 0.87) than we did here. This can be explained by the fact that our study population only comprised healthy volunteers, who all had a normal ABPI index of greater than 100%, and thus a very small range of values, which was not the case with the other parameters measured.

Table 3. Mean values of $TcpO_2$ and NIRS (attached to the lateral side of the calf) in the standing position, before, during and after the treadmill test, mean ABPI (resting and after exercise) and TSBP for the different Fontaine stages

	Treadmill test	Control ($n=16$)	p-value	F2 ($n=20$)	p-value	F3/4 ($n=9$)
ABPI (mmHg)	Before	113	*	83	ns	73
	After	107	*	63	ns	69
TSBP (mmHg)		111	*	79	ns	69
$TcpO_2$ (mmHg)	Before	70	ns	69	*	49
	During	59	ns	54	*	38
	End	64	ns	59	*	42
NIRS (TsO_2; %)	Before	63	ns	58	ns	53
	During	41	*	12	ns	26
	End	60	*	20	ns	29

*$p<0.05$; ns, not significant.

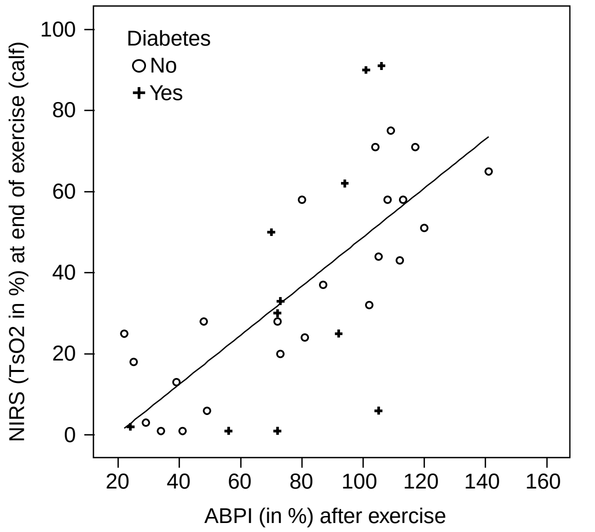

Fig. 2. Scatter plot of the TsO_2 values obtained by NIRS at the calf and the ABPI after exercise. A lower ABPI is significantly correlated with a reduced TsO_2 after exercise ($R=0.73$; $p<0.001$). Diabetic patients show a higher correlation coefficient ($R=0.84$) than non-diabetics ($R=0.60$).

A striking observation was the difference in TsO_2 between men and women as measured on the calf, but not on the dorsum of the foot. A possible explanation may be the structure of the subcutis, which is likely to comprise more adipose tissue in women, as was suggested in an earlier study, or perhaps a better 'muscle fitness' in men.[25] In patients with leg ischaemia this difference between the sexes diminished, probably because arterial insufficiency affects muscle fitness or induces atrophy of skin and subcutis.

NIRS was not able to differentiate between the presence or absence of vascular disease at rest. Apparently, collateral blood flow is initially sufficient to supply the tissues with oxygen. Profound haemoglobin desaturation during exercise was more marked in patients with peripheral vascular disease than in the healthy reference population. These findings are in agreement with earlier studies using other NIRS devices.[13–15,26] Furthermore, they are consistent with the pathogenesis of intermittent claudication, which occurs when the circulation fails to meet oxygen requirements during exercise and explains the good correlation between TsO_2 and ABPI as found after the treadmill exercise. The presence of diabetes mellitus negatively influenced this correlation. Which is understandable as it is known that the ABPI is less reliable in diabetic patients due to media sclerosis of the ankle arteries. In these patients, NIRS may provide a more reliable assessment of the severity of leg ischaemia.[27]

The poor correlation we found between NIRS and ankle blood pressures at rest is in agreement with the findings of Egun *et al.*,[15] whereas in other studies this relationship was more prominent.[13,14,26] The degree of haemoglobin desaturation was significantly greater in claudicants than in healthy subjects, in particular when using the recovery time of TsO_2 after exercise.[13,14] In our study, this parameter could not be assessed accurately due to the design of the measurement protocol, but might offer more possibilities to apply NIRS in diagnosing vascular disease. For this purpose, toe systolic blood pressures measurements are a simple alternative, particularly in diabetes and in identifying critical ischaemia.[28] In our study population, however, TSBP measurements could not differentiate between the various stages of leg ischaemia. This may have been due to the inclusion of relatively few patients with critical leg ischaemia.

As ABPI and TSBP are inexpensive and easy methods to accurately evaluate arterial insufficiency already under resting conditions,[19,28] NIRS appears to have no additional diagnostic value in routine clinical practice. The technique does seem to be suited for application in vascular or other areas of (clinical) research as it can directly measure the local TsO_2 as parameter of interest, for instance in the diagnosis of compartmental syndrome,[5] cerebral ischaemia during

Table 4. Mean values of $TcpO_2$, LDF and NIRS; $TcpO_2$ and NIRS were attached on the dorsum of the foot while NIRS was placed on the hallux

	Provocation test	Control	*p*-value	F2	*p*-value	F3/4
$TcpO_2$	Rest	52	ns	49	ns	33
	Peak-rest	0.6	ns	−1.2	ns	−1.7
	Sup/sit ratio	1.1	ns	1.1	ns	1.1
NIRS	Rest	77	ns	74	ns	67
	Peak-rest	9	ns	6	ns	5
	Sup/sit ratio	1.4	*	1.1	ns	1.1
LDF	Rest	0.059	ns	0.055	ns	0.015
	Peak-rest	0.167	ns	0.182	ns	0.072
	Sup/sit ratio	3.3	ns	2.5	ns	1.1

Parameters of interest were the resting values, peak-rest values (postocclusive hyperaemia response) and the supine/sitting ratios (vasoconstriction response). *$p<0.05$; ns, not significant.

carotid endarterectomy,[7] (patho)physiological studies on changes in energy metabolism,[6] or the effects of exercise training.[3] However, the variability due to the influence of the subcutis and skin perfusion has to be overcome if the intramuscular perfusion per se is to be appreciated.

In conclusion, although NIRS is a reproducible technique to measure tissue or muscle oxygenation, it does not play a convincing role in the routine determination of leg ischaemia, for which simple and reliable methods are readily available. NIRS cannot be regarded as a measure of macrocirculatory or microcirculatory perfusion as measured by the commonly available techniques as used in this study, as it showed no clear similarities with these parameters.

References

1 Jöbsis FF. Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* 1977;**198**(4323):1264–1267.

2 Taylor DE, Simonson SG. Use of near-infrared spectroscopy to monitor tissue oxygenation. *New Horiz* 1996;**4**(4):420–425.

3 Colier WN, Meeuwsen IB, Degens H, Oeseburg B. Determination of oxygen consumption in muscle during exercise using near infrared spectroscopy. *Acta Anaesthesiol Scand* 1995;**107**:151–155.

4 Comerota AJ, Throm RC, Kelly P, Jaff M. Tissue (muscle) oxygen saturation (StO2): a new measure of symptomatic lower-extremity arterial disease. *J Vasc Surg* 2003;**38**(4):724–729.

5 Giannotti G, Cohn SM, Brown M, Varela JE, McKenny MG, Wiseberg JA. Utility of near-infrared spectroscopy in the diagnosis of lower extremity compartment syndrome. *J Trauma* 2000;**48**:396–401.

6 Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P *et al*. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. *Circulation* 2003;**107**:3040–3046.

7 Mille T, Tachimiri ME, Klersy C, Ticozzelli G, Bellinzona G, Blangetti I *et al*. Near infrared spectroscopy monitoring during carotid endarterectomy: which threshold value is critical? *Eur J Vasc Endovasc Surg* 2004;**27**(6):646–650.

8 Kragsterman B, Parsson H, Bergqvist D. Local haemodynamic changes during carotid endarterectomy—the influence on cerebral oxygenation. *Eur J Vasc Endovasc Surg* 2004;**27**(4):398–402.

9 Williams IM, Mortimer AJ, McCollum CN. Recent developments in cerebral monitoring—near-infrared light spectroscopy. An overview. *Eur J Vasc Endovasc Surg* 1996;**12**(3):263–271.

10 Cheatle TR, Potter LA, Cope M, Delpy DT, Coleridge Smith PD, Scurr JH. Near-infrared spectroscopy in peripheral vascular disease. *Br J Surg* 1991;**78**(4):405–408.

11 McCully KK, Halber C, Posner JD. Exercise-induced changes in oxygen saturation in the calf muscles of elderly subjects with peripheral vascular disease. *J Gerontol* 1994;**49**(3):B128–B134.

12 Komiyama T, Shigematsu H, Yasuhara H, Muto T. An objective assessment of intermittent claudication by near-infrared spectroscopy. *Eur J Vasc Surg* 1994;**8**(3):294–296.

13 Kooijman HM, Hopman MT, Colier WN, van der Vliet JA, Oeseburg B. Near infrared spectroscopy for noninvasive assessment of claudication. *J Surg Res* 1997;**72**(1):1–7.

14 Seifalian AM, Atwal A, White S, Mikhailidis DP, Baker D, Hamilton G. A role for near infrared spectroscopy in the assessment of intermittent claudication. *Int Angiol* 2001;**20**(4): 301–306.

15 Egun A, Farooq V, Torella F, Cowley R, Thorniley MS, McCollum CN. The severity of muscle ischemia during intermittent claudication. *J Vasc Surg* 2002;**36**(1):89–93.

16 Ubbink DT, Jacobs MJ, Tangelder GJ, Slaaf DW, Reneman RS. The usefulness of capillary microscopy, transcutaneous oximetry and laser Doppler fluxmetry in the assessment of the severity of lower limb ischaemia. *Int J Microcirc Clin Exp* 1994;**14**(1–2):34–44.

17 Ubbink DT, Jacobs MJ. The significance of microcirculatory examinations. *Eur J Vasc Endovasc Surg* 1998;**16**(5):373–376.

18 De Graaff JC, Ubbink DT, Legemate DA, de Haan RJ, Jacobs MJ. The usefulness of a laser Doppler in the measurement of toe blood pressures. *J Vasc Surg* 2000;**32**(6):1172–1179.

19 Ubbink DT, Tulevski II, de Graaff JC, Legemate DA, Jacobs MJ. Optimisation of the non-invasive assessment of critical limb ischaemia requiring invasive treatment. *Eur J Vasc Endovasc Surg* 2000;**19**(2):131–137.

20 De Graaff JC, Ubbink DT, Legemate DA, de Haan RJ, Jacobs MJ. Interobserver and intraobserver reproducibility of peripheral blood and oxygen pressure measurements in the assessment of lower extremity arterial disease. *J Vasc Surg* 2001; **33**(5):1033–1040.

21 Caruana MF, Bradbury AW, Adam DJ. The validity, reliability, reproducibility and extended utility of ankle to brachial pressure index in current vascular surgical practice. *Eur J Vasc Endovasc Surg* 2005;**29**(5):443–451.

22 Lubbers DW. Theoretical basis of the transcutaneous blood gas measurements. *Crit Care Med* 1981;**9**:721–733.

23 Fleiss JI, Cohen RA. The equivalent of weighted kappa and the intraclass correlation coefficient as measures for reliability. *Educ Psychol Meas* 1973;**33**:613–619.

24 Kragelj R, Jarm T, Miklavcic D. Reproducibility of parameters of postocclusive reactive hyperaemia measured by near infrared spectroscopy and transcutaneous oximetry. *Ann Biomed Eng* 2000; **28**(2):168–173.

25 Matsushita K, Homma S, Okada E. Influence of adipose tissue on muscle oxygenation measurement with NIRS instrument. *SPIE* 1998;**3194**:159–165.

26 Komiyama T, Onozuka A, Miyata T, Shigematsu H. Oxygen saturation measurement of calf muscle during exercise in intermittent claudication. *EurJ Vasc Endovasc Surg* 2002;**23**(5): 388–392.

27 Komiyama T, Shigematsu H, Yashara H, Muto T. Near-infrared spectroscopy grades the severity of intermittent claudication in diabetics more accurately than ankle pressure measurements. *Br J Surg* 2000;**87**(4):459–466.

28 Apelqvist J, Castenfors J, Larsson J, Stenstrom A, Agardh CD. Prognostic value of systolic ankle and toe blood pressure levels in outcome of diabetic foot ulcer. *Diab Care* 1989; **12**:373–378.

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